

**WHAT IS CLAIMED IS:**

1. An anti-CD20 antibody-cytotoxic agent conjugate, wherein the cytotoxic agent of the anti-CD20 antibody-cytotoxic agent conjugate has an  $IC_{50}$  of between 40-fold and 4,000-fold less than the  $IC_{50}$  of doxorubicin, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, and wherein the  $IC_{50}$  of each of the cytotoxic agent and doxorubicin is measured by a method comprising:
  - (a) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the cytotoxic agent for a 72- to 96-hour period;
  - (b) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of doxorubicin for a 72- to 96-hour period; and
  - (c) identifying a concentration of the cytotoxic agent and doxorubicin, respectively, at which 50% fewer cells in the CD20-expressing cell populations of steps (a) and (b), respectively, are viable at the end of the period relative to a CD20-expressing cell population cultured in the absence of the cytotoxic agent and doxorubicin,wherein the CD20-expressing cell populations of steps (a), (b) and (c) are of the same cell type and are cultured under the same conditions, and wherein the concentration of the cytotoxic agent and doxorubicin identified in step (c) is the  $IC_{50}$  of the cytotoxic agent and doxorubicin, respectively.
2. The conjugate of claim 1, wherein the  $IC_{50}$  of the cytotoxic agent is between 100-fold and 1000-fold less than the  $IC_{50}$  of doxorubicin.
3. The conjugate of claim 1, wherein the  $IC_{50}$  of the cytotoxic agent is between 50-fold and 200-fold less than the  $IC_{50}$  of doxorubicin.
4. The conjugate of claim 1, wherein the  $IC_{50}$  of the cytotoxic agent is between 400-fold and 600-fold less than the  $IC_{50}$  of doxorubicin.
5. The conjugate of claim 1, wherein the  $IC_{50}$  of the cytotoxic agent is between 800-fold and 1200-fold less than the  $IC_{50}$  of doxorubicin.
6. An anti-CD20 antibody-cytotoxic agent conjugate, wherein the anti-CD20 antibody-cytotoxic agent conjugate has an  $IC_{50}$  of between 40-fold and 4,000-fold less than the  $IC_{50}$  of an anti-CD20 antibody-doxorubicin conjugate, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, and wherein the  $IC_{50}$  of each of the anti-CD20 antibody-cytotoxic agent

conjugate and the anti-CD20 antibody-doxorubicin conjugate is measured by a method comprising:

- 5 (a) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the anti-CD20 antibody-cytotoxic agent conjugate for a 72- to 96-hour period;
- (b) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the anti-CD20 antibody-doxorubicin conjugate for a 72- to 96-hour period; and
- 10 (c) identifying a concentration of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate, respectively, at which 50% fewer cells in the CD20-expressing cell populations of steps (a) and (b), respectively, are viable at the end of the period relative to a CD20-expressing cell population type cultured in the absence of the anti-CD20 antibody-cytotoxic agent  
15 conjugate and the anti-CD20 antibody-doxorubicin conjugate, wherein the CD20-expressing cell populations of steps (a), (b) and (c) are of the same cell type and are cultured under the same conditions, and wherein the concentration of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate identified in step (c) is the  
20  $IC_{50}$  of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate, respectively.

7. The conjugate of claim 6, wherein the  $IC_{50}$  of the anti-CD20 antibody-cytotoxic agent conjugate is between 100-fold and 1000-fold less than the  $IC_{50}$  of the anti-CD20 antibody-doxorubicin conjugate.

25 8. The conjugate of claim 6, wherein the  $IC_{50}$  of the anti-CD20 antibody-cytotoxic agent conjugate is at least 50-fold and 200-fold less than the  $IC_{50}$  of the anti-CD20 antibody-doxorubicin conjugate.

9. The conjugate of claim 6, wherein the  $IC_{50}$  of the anti-CD20 antibody-cytotoxic agent conjugate is between 400-fold and 600-fold less than the  $IC_{50}$  of the anti-  
30 CD20 antibody-doxorubicin conjugate.

10. The conjugate of claim 6, wherein the  $IC_{50}$  of the anti-CD20 antibody-cytotoxic agent conjugate is at least 800-fold and 1200-fold less than the  $IC_{50}$  of the anti-CD20 antibody-doxorubicin conjugate.

11. The conjugate of claim 1 or 6, wherein the CD20-expressing cell population is a population of Daudi cells, Ramos cells, Raji cells, IM-9 cells, HS-Sultan cells, ARH-77 cells, HT cells, RL cells, DB cells, or 295R cells.

12. An anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, wherein the rates of accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the unconjugated antibody, wherein the populations of steps (a) and (b) are cultured under the same conditions; and
- (c) measuring the amount of the conjugate and unconjugated antibody accumulated in the populations of steps (a) and (b), respectively.

13. The conjugate of claim 12, wherein the rates of accumulation of the conjugate and the unconjugated form of the antibody in the CD20-expressing cell are determined by:

- (a) culturing a population of the CD20-expressing cell in the presence of the conjugate, wherein the antibody portion of the conjugate is labeled with a radioactive isotope;
- (b) culturing a population of the CD20-expressing cell with the unconjugated form of the antibody under the same conditions as the culturing of step (a), wherein the unconjugated form of the antibody is labeled with the radioactive isotope;
- (c) washing each of the populations of steps (a) and (b) under acidic conditions; and
- (d) comparing the amount of the radioactive isotope in the populations of steps (a) and (b) after the washing of step (c),

wherein the rate of accumulation of the conjugate in the CD20-expressing cell is between 20-fold and 5,000-fold greater than the rate of accumulation of the unconjugated form of the anti-CD20 antibody in the CD20-expressing cell if the amount of the radioactive isotope in the population of step (a) is between 20-fold and 5,000-fold greater than the amount of the radioactive isotope in the population of step (b).

14. The conjugate of claim 12, wherein the CD20-expressing cell is a Daudi cell, a Ramos cell, a Raji cell, an IM-9 cell, a HS-Sultan cell, an ARH-77 cell, a HT cell, a RL cell, a DB cell, or a 295R cell.

15. The conjugate of claim 12, wherein the conjugate has a rate of accumulation inside the CD20-expressing cell that is between 50-fold and 2,500-fold greater than the rate of accumulation inside the CD20-expressing cell of the anti-CD20 antibody in unconjugated form.

16. The conjugate of claim 12, wherein the conjugate has a rate of accumulation inside the CD20-expressing cell that is between 100-fold and 1,000-fold greater than the rate of accumulation inside the CD20-expressing cell of the anti-CD20 antibody in unconjugated form.

17. The conjugate of claim 12, wherein the conjugate has a rate of accumulation inside the CD20-expressing cell that is between 25-fold and 75-fold greater than the rate of accumulation inside the CD20-expressing cell of the anti-CD20 antibody in unconjugated form.

18. An anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an anti-CD20 antibody-doxorubicin conjugate in a CD20-expressing cell of the same cell type, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, and wherein the rates of accumulation of the anti-CD20 antibody-cytotoxic agent conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-cytotoxic agent conjugate;
- (b) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate, wherein the populations of steps (a) and (b) are cultured under the same conditions; and
- (c) measuring the amount of the anti-CD20 antibody-cytotoxic agent conjugate and anti-CD20 antibody-doxorubicin conjugate accumulated in the populations of steps (a) and (b), respectively.

19. The anti-CD20 antibody-cytotoxic agent conjugate of claim 18, wherein the conjugate has a rate of accumulation inside the CD20-expressing cell that is between 50-fold and 2,500-fold greater than the rate of accumulation inside the CD20-expressing cell of the anti-CD20 antibody-doxorubicin conjugate.

20. The anti-CD20 antibody-cytotoxic agent conjugate of claim 18, wherein the conjugate has a rate of accumulation inside the CD20-expressing cell that is between 100-fold and 1,000-fold greater than the rate of accumulation inside the CD20-expressing cell of the anti-CD20 antibody-doxorubicin conjugate.

21. The anti-CD20 antibody-cytotoxic agent conjugate of claim 18, wherein the conjugate has a rate of accumulation inside the CD20-expressing cell that is between 25-fold and 75-fold greater than the rate of accumulation inside the CD20-expressing cell of the anti-CD20 antibody-doxorubicin conjugate.

22. An anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, wherein the accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the unconjugated form of the anti-CD20 antibody; and
- (c) detecting by confocal fluorescence microscopy localization of the conjugate and the unconjugated form of the anti-CD20 antibody in the populations of steps (a) and (b), respectively,

wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the CD20-expressing cell if:

- (i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the unconjugated form of the antibody in a non-peripheral region; or
- (ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the majority of CD20-expressing cells of the population of step (b).

23. The conjugate of claim 22, wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 5-fold and 2,500-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the CD20-expressing cell.

5 24. The conjugate of claim 22, wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 50-fold and 1,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the CD20-expressing cell.

10 25. The conjugate of claim 22, wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 100-fold and 500-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the CD20-expressing cell.

15 26. An anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an anti-CD20 antibody-doxorubicin conjugate, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, in the CD20-expressing cell, wherein the accumulation of the conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- 20 (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate; and
- 25 (c) detecting by confocal fluorescence microscopy localization of the conjugate and the anti-CD20 antibody-doxorubicin conjugate in the populations of steps (a) and (b), respectively,

wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the CD20-expressing cell if:

- 35 (i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the anti-CD20 antibody-doxorubicin conjugate in a non-peripheral region; or

(ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the majority of CD20-expressing cells of the population of step (b).

27. The conjugate of claim 26, wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 5-fold and 2,500-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the CD20-expressing cell.

28. The conjugate of claim 26, wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 50-fold and 1,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the CD20-expressing cell.

29. The conjugate of claim 26, wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 100-fold and 500-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the CD20-expressing cell.

30. The conjugate of claim 22 or 26, wherein said majority of CD20-expressing cells of the population of step (b) is at least 60% of the cells in the population.

31. The conjugate of claim 22 or 26, wherein said majority of CD20-expressing cells of the population of step (b) is at least 70% of the cells in the population.

32. The conjugate of claim 22 or 26, wherein said majority of CD20-expressing cells of the population of step (b) is at least 80% of the cells in the population.

33. The conjugate of claim 22 or 26, wherein the CD20-expressing cell is a Daudi cell, a Ramos cell, a Raji cell, an IM-9 cell, a HS-Sultan cell, an ARH-77 cell, a HT cell, a RL cell, a DB cell, or a 295R cell.

34. The conjugate of claim 6, 18, or 26, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the conjugate of the anti-CD20 antibody and doxorubicin comprise the same linker.

35. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the cytotoxic agent is selected from the group consisting of an enediyne, a lexitropsin, a duocarmycin, a taxane, a puromycin, a dolastatin, a maytansinoid, a DNA minor groove binding agent, a DNA minor groove alkylating agent, and a vincaalkaloid.

36. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the cytotoxic agent is paclitaxel, docetaxel, CC-1065, SN-38, topotecan, morpholino-doxorubicin, rhizoxin,

cyanomorpholino-doxorubicin, dolastatin-10, echinomycin, combretastatin, calicheamicin, maytansine, DM-1, auristatin E, auristatin EB, auristatin E-FP, monomethyl auristatin E, or netropsin.

37. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the cytotoxic agent is an anti-tubulin agent.

38. The conjugate of claim 37, wherein the cytotoxic agent is selected from the group consisting of a vinca alkaloid, a podophyllotoxin, a taxane, a baccatin derivative, a cryptophysin, a maytansinoid, a combretastatin, and a dolastatin.

39. The conjugate of claim 37, wherein the cytotoxic agent is vincristine, vinblastine, vindesine, vinorelbine, VP-16, camptothecin, paclitaxel, docetaxel, epithilone A, epithilone B, nocodazole, colchicine, colcimid, estramustine, cemadotin, discodermolide, maytansine, DM-1, auristatin E, auristatin EB, auristatin E-FP, monomethyl auristatin E, or eleutherobin.

40. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the cytotoxic agent is monomethyl auristatin E.

41. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20 antibody is conjugated to the cytotoxic agent via a peptide linker.

42. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20 antibody is conjugated to the cytotoxic agent via a val-cit linker or a phe-lys linker.

43. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20 antibody is conjugated to the cytotoxic agent via a hydrazone-linker, or a disulfide-linker.

44. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the conjugate is Rituximab-val-cit-monomethyl auristatin E.

45. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20 antibody is conjugated to the cytotoxic agent via a linker that is hydrolyzable at a pH of less than 5.5.

46. The conjugate of claim 45, wherein the linker is hydrolyzable at a pH of less than 5.0.

47. The conjugate of claim 45, wherein the linker is a hydrazone linker or a disulfide linker.

48. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20 antibody is conjugated to the cytotoxic agent via a linker, wherein the linker is cleavable by a protease.



49. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20 antibody is conjugated to the cytotoxic agent via a peptide linker, and wherein the linker is cleavable by a protease.
- 5 50. The conjugate of claim 48, wherein the protease is a membrane-associated protease.
51. The conjugate of claim 48, wherein the protease is an intracellular protease.
52. The conjugate of claim 48, wherein the protease is an endosomal protease.
53. The conjugate of claim 48, wherein the protease is a lysosomal protease.
54. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20  
10 antibody is a monoclonal antibody, a chimeric antibody, a human antibody, a humanized antibody, a glycosylated antibody, a multispecific antibody, a human antibody, a single-chain antibody, a Fab fragment, a F(ab') fragment, a F(ab')<sub>2</sub> fragment, a Fd, a single-chain Fv, a disulfide-linked Fv, a fragment comprising a V<sub>L</sub> domain, a polypeptide that binds specifically to CD20, or a fragment comprising a V<sub>H</sub> domain.
- 15 55. The conjugate of claim 54, wherein the anti-CD20 antibody is a human antibody.
56. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20 antibody is a bispecific antibody.
57. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20  
20 antibody is not a bispecific antibody.
58. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20 antibody is conjugated to a radioisotope.
59. The conjugate of claim 58, wherein the radioisotope is selected from the group consisting of <sup>90</sup>yttrium, <sup>111</sup>indium, <sup>211</sup>astatide, <sup>131</sup>iodine, <sup>213</sup>bismuth, and <sup>225</sup>actinium.
- 25 60. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20 antibody comprises one or more CDRs of C2B8, 1F5, FB1, 2H7, 93-1B3, 109-3C2, B1, B9E9, 7D1, H147, L26, L27, or MEM97.
61. The conjugate of claim 60, wherein the anti-CD20 antibody is a humanized antibody.
- 30 62. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20 antibody comprises the variable region of C2B8, 1F5, FB1, 2H7, 93-1B3, 109-3C2, B1, B9E9, 7D1, H147, L26, L27, or MEM97.
63. The conjugate of claim 62, wherein the anti-CD20 antibody is a chimeric antibody.

64. The conjugate of claim 1, 6, 12, 18, 22, or 26, wherein the anti-CD20 antibody is an affinity matured variant of C2B8, 1F5, FB1, 2H7, 93-1B3, 109-3C2, B1, B9E9, 7D1, H147, L26, L27, or MEM97.

65. A pharmaceutical composition comprising an anti-CD20 antibody-cytotoxic agent conjugate, wherein the cytotoxic agent of the anti-CD20 antibody-cytotoxic agent conjugate has an  $IC_{50}$  of between 40-fold and 4,000-fold less than the  $IC_{50}$  of doxorubicin, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, and wherein the  $IC_{50}$  of each of the cytotoxic agent and doxorubicin is measured by a method comprising:

- (a) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the cytotoxic agent for a 72- to 96-hour period;
  - (b) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of doxorubicin for a 72- to 96-hour period; and
  - (c) identifying a concentration of the cytotoxic agent and doxorubicin, respectively, at which 50% fewer cells in the CD20-expressing cell populations of steps (a) and (b), respectively, are viable at the end of the period relative to a CD20-expressing cell population cultured in the absence of the cytotoxic agent and doxorubicin,
- wherein the CD20-expressing cell populations of steps (a), (b) and (c) are of the same cell type and are cultured under the same conditions, and wherein the concentration of the cytotoxic agent and doxorubicin identified in step (c) is the  $IC_{50}$  of the cytotoxic agent and doxorubicin, respectively.

66. A pharmaceutical composition comprising an anti-CD20 antibody-cytotoxic agent conjugate, wherein the anti-CD20 antibody-cytotoxic agent conjugate has an  $IC_{50}$  of between 40-fold and 4,000-fold less than the  $IC_{50}$  of an anti-CD20 antibody-doxorubicin conjugate, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, and wherein the  $IC_{50}$  of each of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate is measured by a method comprising:

- (a) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the anti-CD20 antibody-cytotoxic agent conjugate for a 72- to 96-hour period;

(b) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the anti-CD20 antibody-doxorubicin conjugate for a 72- to 96-hour period; and

(c) identifying a concentration of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate, respectively, at which 50% fewer cells in the CD20-expressing cell populations of steps (a) and (b), respectively, are viable at the end of the period relative to a CD20-expressing cell population cultured in the absence of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate,

wherein the CD20-expressing cell populations of steps (a), (b) and (c) are of the same cell type and are cultured under the same conditions,

and wherein the concentration of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate identified in step (c) is the IC<sub>50</sub> of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate, respectively.

67. A pharmaceutical composition comprising an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the rates of accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

(a) culturing a population of the CD20-expressing cell with the conjugate;

(b) culturing a population of the CD20-expressing cell with the unconjugated antibody, wherein the populations of steps (a) and (b) are cultured under the same conditions; and

(c) measuring the amount of the conjugate and unconjugated antibody accumulated in the populations of steps (a) and (b), respectively.

68. A pharmaceutical composition comprising an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an anti-CD20 antibody-doxorubicin conjugate in a CD20-expressing cell of the same cell type, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20

antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, and wherein the rates of accumulation of the anti-CD20 antibody-cytotoxic agent conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- 5                   (a)     culturing a population of the CD20-expressing cell with the anti-CD20 antibody-cytotoxic agent conjugate;
- (b)     culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate, wherein the populations of steps (a) and (b) are cultured under the same conditions; and
- 10               (c)     measuring the amount of the anti-CD20 antibody-cytotoxic agent conjugate and anti-CD20 antibody-doxorubicin conjugate accumulated in the populations of steps (a) and (b), respectively.

69.     A pharmaceutical composition comprising an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate exhibits an accumulation in a non-peripheral region  
15     inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

- 20               (a)     culturing a population of the CD20-expressing cell with the conjugate;
- (b)     culturing a population of the CD20-expressing cell with the unconjugated form of the anti-CD20 antibody; and
- 25               (c)     detecting by confocal fluorescence microscopy localization of the conjugate and the unconjugated form of the anti-CD20 antibody in the populations of steps (a) and (b), respectively,

              wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater  
30     than the accumulation of the unconjugated form of the anti-CD20 antibody in the CD20-expressing cell if:

- (i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the unconjugated  
35     form of the antibody in a non-peripheral region; or

(ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the majority of CD20-expressing cells of the population of step (b).

70. A pharmaceutical composition comprising an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an anti-CD20 antibody-doxorubicin conjugate, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, in the CD20-expressing cell, wherein the accumulation of the conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate; and
- (c) detecting by confocal fluorescence microscopy localization of the conjugate and the anti-CD20 antibody-doxorubicin conjugate in the populations of steps (a) and (b), respectively,

wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the CD20-expressing cell if:

- (i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the anti-CD20 antibody-doxorubicin conjugate in a non-peripheral region; or
- (ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the majority of CD20-expressing cells of the population of step (b).

71. A method of treating a CD20-expressing cancer, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the cytotoxic agent of the anti-CD20 antibody-cytotoxic agent conjugate has an  $IC_{50}$  of between 40-fold and 4,000-fold less than the  $IC_{50}$  of doxorubicin, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, and wherein the  $IC_{50}$  of each of the cytotoxic agent and doxorubicin is measured by a method comprising:

- (a) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the cytotoxic agent for a 72- to 96-hour period;
  - (b) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of doxorubicin for a 72- to 96-hour period; and
  - (c) identifying a concentration of the cytotoxic agent and doxorubicin, respectively, at which 50% fewer cells in the CD20-expressing cell populations of steps (a) and (b), respectively, are viable at the end of the period relative to a CD20-expressing cell population cultured in the absence of the cytotoxic agent and doxorubicin,
- wherein the CD20-expressing cell populations of steps (a), (b) and (c) are of the same cell type and are cultured under the same conditions,
- and wherein the concentration of the cytotoxic agent and doxorubicin identified in step (c) is the  $IC_{50}$  of the cytotoxic agent and doxorubicin, respectively.

72. A method of treating a CD20-expressing cancer, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the anti-CD20 antibody-cytotoxic agent conjugate has an  $IC_{50}$  of between 40-fold and 4,000-fold less than the  $IC_{50}$  of an anti-CD20 antibody-doxorubicin conjugate, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, and wherein the  $IC_{50}$  of each of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate is measured by a method comprising:

- (a) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the anti-CD20 antibody-cytotoxic agent conjugate for a 72- to 96-hour period;

- (b) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the anti-CD20 antibody-doxorubicin conjugate for a 72- to 96-hour period; and
- (c) identifying a concentration of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate, respectively, at which 50% fewer cells in the CD20-expressing cell populations of steps (a) and (b), respectively, are viable at the end of the period relative to a CD20-expressing cell population cultured in the absence of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate,
- wherein the CD20-expressing cell populations of steps (a), (b) and (c) are of the same cell type and are cultured under the same conditions,
- and wherein the concentration of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate identified in step (c) is the  $IC_{50}$  of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate, respectively.

73. A method of treating a CD20-expressing cancer, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the rates of accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the unconjugated antibody, wherein the populations of steps (a) and (b) are cultured under the same conditions; and
- (c) measuring the amount of the conjugate and unconjugated antibody accumulated in the populations of steps (a) and (b), respectively.

74. A method of treating a CD20-expressing cancer, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an anti-

CD20 antibody-doxorubicin conjugate in a CD20-expressing cell of the same cell type, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, and wherein the rates of accumulation of the anti-  
5 CD20 antibody-cytotoxic agent conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-cytotoxic agent conjugate;
- (b) culturing a population of the CD20-expressing cell with the anti-  
10 CD20 antibody-doxorubicin conjugate, wherein the populations of steps (a) and (b) are cultured under the same conditions; and
- (c) measuring the amount of the anti-CD20 antibody-cytotoxic agent conjugate and anti-CD20 antibody-doxorubicin conjugate accumulated in the populations of steps (a) and (b), respectively.

15 75. A method of treating a CD20-expressing cancer, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing  
20 cell, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the  
25 unconjugated form of the anti-CD20 antibody; and
- (c) detecting by confocal fluorescence microscopy localization of the conjugate and the unconjugated form of the anti-CD20 antibody in the populations of steps (a) and (b), respectively,

30 wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the CD20-expressing cell if:



(i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the unconjugated form of the antibody in a non-peripheral region; or

(ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the majority of CD20-expressing cells of the population of step (b).

76. A method of treating a CD20-expressing cancer, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an anti-CD20 antibody-doxorubicin conjugate, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, in the CD20-expressing cell, wherein the accumulation of the conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

(a) culturing a population of the CD20-expressing cell with the conjugate;

(b) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate; and

(c) detecting by confocal fluorescence microscopy localization of the conjugate and the anti-CD20 antibody-doxorubicin conjugate in the populations of steps (a) and (b), respectively,

wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the CD20-expressing cell if:

(i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the anti-CD20 antibody-doxorubicin conjugate in a non-peripheral region; or

(ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the majority of CD20-expressing cells of the population of step (b).

77. The method of claim 71, 72, 73, 74, 75 or 76, wherein the cancer is a follicular Non-Hodgkin's Lymphoma, a small lymphocytic lymphoma, a chronic lymphocytic leukemia, a lymphoplasmacytic Non-Hodgkin's Lymphoma, a hairy cell leukemia, a B cell prolymphocytic leukemia, a CD20-positive Acute lymphocytic leukemia, or a marginal zone Non-Hodgkin's Lymphoma.

78. A method of treating an immune disorder involving CD20-expressing cells, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the cytotoxic agent of the anti-CD20 antibody-cytotoxic agent conjugate has an  $IC_{50}$  of between 40-fold and 4,000-fold less than the  $IC_{50}$  of doxorubicin, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, and wherein the  $IC_{50}$  of each of the cytotoxic agent and doxorubicin is measured by a method comprising:

- (a) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the cytotoxic agent for a 72- to 96-hour period;
- (b) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of doxorubicin for a 72- to 96-hour period; and
- (c) identifying a concentration of the cytotoxic agent and doxorubicin, respectively, at which 50% fewer cells in the CD20-expressing cell populations of steps (a) and (b), respectively, are viable at the end of the period relative to a CD20-expressing cell population cultured in the absence of the cytotoxic agent and doxorubicin,

wherein the CD20-expressing cell populations of steps (a), (b) and (c) are of the same cell type and are cultured under the same conditions,

and wherein the concentration of the cytotoxic agent and doxorubicin identified in step (c) is the  $IC_{50}$  of the cytotoxic agent and doxorubicin, respectively.

79. A method of treating an immune disorder involving CD20-expressing cells, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the anti-CD20 antibody-cytotoxic

agent conjugate has an  $IC_{50}$  of between 40-fold and 4,000-fold less than the  $IC_{50}$  of an anti-CD20 antibody-doxorubicin conjugate, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, and  
5 wherein the  $IC_{50}$  of each of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate is measured by a method comprising:

- (a) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the anti-CD20 antibody-cytotoxic agent conjugate for a 72- to 96-hour period;
- 10 (b) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the anti-CD20 antibody-doxorubicin conjugate for a 72- to 96-hour period; and
- (c) identifying a concentration of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate,  
15 respectively, at which 50% fewer cells in the CD20-expressing cell populations of steps (a) and (b), respectively, are viable at the end of the period relative to a CD20-expressing cell population cultured in the absence of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate,

20 wherein the CD20-expressing cell populations of steps (a), (b) and (c) are of the same cell type and are cultured under the same conditions,

and wherein the concentration of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate identified in step (c) is the  $IC_{50}$  of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate, respectively.  
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80. A method of treating an immune disorder involving CD20-expressing cells, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than  
30 the rate of accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, and wherein the rates of accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the  
35 conjugate;

- (b) culturing a population of the CD20-expressing cell with the unconjugated antibody, wherein the populations of steps (a) and (b) are cultured under the same conditions; and
- (c) measuring the amount of the conjugate and unconjugated antibody accumulated in the populations of steps (a) and (b), respectively.

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81. A method of treating an immune disorder involving CD20-expressing cells, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an anti-CD20 antibody-doxorubicin conjugate in a CD20-expressing cell of the same cell type, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, and wherein the rates of accumulation of the anti-CD20 antibody-cytotoxic agent conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

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- (a) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-cytotoxic agent conjugate;
- (b) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate, wherein the populations of steps (a) and (b) are cultured under the same conditions; and
- (c) measuring the amount of the anti-CD20 antibody-cytotoxic agent conjugate and anti-CD20 antibody-doxorubicin conjugate accumulated in the populations of steps (a) and (b), respectively.

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82. A method of treating an immune disorder involving CD20-expressing cells, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

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- (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the unconjugated form of the anti-CD20 antibody; and

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(c) detecting by confocal fluorescence microscopy localization of the conjugate and the unconjugated form of the anti-CD20 antibody in the populations of steps (a) and (b), respectively,

wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the CD20-expressing cell if:

- (i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the unconjugated form of the antibody in a non-peripheral region; or
- (ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the majority of CD20-expressing cells of the population of step (b).

83. A method of treating an immune disorder involving CD20-expressing cells, comprising administering to a subject in need of such treatment an effective amount of an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an anti-CD20 antibody-doxorubicin conjugate, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, in the CD20-expressing cell, wherein the accumulation of the conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate; and
- (c) detecting by confocal fluorescence microscopy localization of the conjugate and the anti-CD20 antibody-doxorubicin conjugate in the populations of steps (a) and (b), respectively,

wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the CD20-expressing cell if:

- (i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the anti-CD20 antibody-doxorubicin conjugate in a non-peripheral region; or
- (ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the majority of CD20-expressing cells of the population of step (b).

84. The method of claim 78, 79, 80, 81, 82, or 83, wherein said immune disorder is rheumatoid arthritis, multiple sclerosis, endocrine ophthalmopathy, systemic lupus erythematosus, myasthenia gravis, Grave's disease, glomerulonephritis, anaphylaxis, allergic reaction, Sjogren's syndrome, juvenile onset (Type I) diabetes mellitus, primary biliary cirrhosis, Wegener's granulomatosis, inflammatory bowel disease, polymyositis, dermatomyositis, Schmidt's syndrome, Addison's disease, adrenalitis, thyroiditis, Hashimoto's thyroiditis, autoimmune thyroid disease, pernicious anemia, chronic hepatitis, lupoid hepatitis, atherosclerosis, demyelinating diseases, subacute cutaneous lupus erythematosus, hypoparathyroidism, autoimmune thrombocytopenia, idiopathic thrombocytopenic purpura, hemolytic anemia, pemphigus vulgaris, pemphigus, dermatitis herpetiformis, alopecia areata, pemphigoid, scleroderma, progressive systemic sclerosis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), adult onset diabetes mellitus (Type II diabetes), ulcerative colitis, Crohn's disease, mixed connective tissue disease, polyarteritis nodosa, systemic necrotizing vasculitis, juvenile onset rheumatoid arthritis, atopic rhinitis, Goodpasture's syndrome, asthma, anti-phospholipid syndrome, farmer's lung, erythema multiforme, autoimmune chronic active hepatitis, bird-fancier's lung, allergic encephalomyelitis, toxic epidermal necrolysis, alveolitis, allergic alveolitis, fibrosing alveolitis, erythema nodosum, transfusion reaction, Caplan's syndrome, erythroblastosis fetalis, Felty's syndrome, IgA nephropathy, Henoch-Schonlein purpura, graft versus host disease, transplantation rejection, relapsing polychondritis, cryoglobulinemia,

Waldenstrom's macroglobulemia, Epstein-Barr virus infection, autoimmune gonadal failure, non-cancerous lymphocytosis, and pre-cancerous lymphocytosis.

85. The method of claim 71, 72, 73, 74, 75, 76, 78, 79, 80, 81, 82, or 83, wherein the method further comprises administering to the subject a second cytostatic or cytotoxic agent.

86. The method of claim 85, wherein the second cytostatic or cytotoxic agent is selected from the group consisting of an alkylating agent, an anthracycline, an antibiotic, an antifolate, an antimetabolite, an antitubulin agent, an auristatin, a chemotherapy sensitizer, a DNA minor groove binder, a DNA replication inhibitor, a duocarmycin, an etoposide, a fluorinated pyrimidine, a lexitropsin, a nitrosourea, a platinol, a purine antimetabolite, a puromycin, a radiation sensitizer, a steroid, a taxane, a topoisomerase inhibitor, a vinca alkaloid, a purine antagonist, and a dihydrofolate reductase inhibitor.

87. The method of claim 85, wherein the second cytostatic or cytotoxic agent is androgen, anthramycin (AMC), asparaginase, 5-azacytidine, azathioprine, bleomycin, busulfan, buthionine sulfoximine, camptothecin, carboplatin, carmustine (BSNU), CC-1065, chlorambucil, cisplatin, colchicine, cyclophosphamide, cytarabine, cytidine arabinoside, cytochalasin B, dacarbazine, dactinomycin (formerly actinomycin), daunorubicin, decarbazine, docetaxel, doxorubicin, an estrogen, 5-fluorodeoxyuridine, 5-fluorouracil, gramicidin D, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine (CCNU), mechlorethamine, melphalan, 6-mercaptopurine, methotrexate, mithramycin, mitomycin C, mitoxantrone, nitroimidazole, paclitaxel, plicamycin, procarbazine, streptozotocin, tenoposide, 6-thioguanine, thioTEPA, topotecan, vinblastine, vincristine, vinorelbine, VP-16, VM-26, azathioprine, mycophenolate mofetil, methotrexate, acyclovir, gangcyclovir, zidovudine, vidarabine, ribavarin, azidothymidine, cytidine arabinoside, amantadine, dideoxyuridine, iododeoxyuridine, poscarnet, or trifluridine.

88. The method of claim 71, 72, 73, 74, 75, or 76, wherein the method further comprises administering to the subject a second antibody that binds to an antigen of the CD20-expressing cancer, and wherein the second antibody is not an anti-CD20 antibody.

89. The method of claim 88, wherein the second antibody is selected from the group consisting of an anti-CD19 antibody, an anti-CD22 antibody, an anti-CD30 antibody, and an anti-CD40 antibody.

90. The method of claim 88, wherein the second antibody is conjugated to a second cytostatic or cytotoxic agent.

91. The method of claim 90, wherein the second cytostatic or cytotoxic agent is a chemotherapeutic agent, a radioisotope or a toxin.

92. The method of claim 71, 72, 73, 74, 75, 76, 78, 79, 80, 81, 82, or 83, wherein the subject is a mammal.

93. The method of claim 71, 72, 73, 74, 75, 76, 78, 79, 80, 81, 82, or 83, wherein the subject is human.

5 94. The method of claim 78, 79, 80, 81, 82, or 83, wherein the method further comprises administering to the subject an immunosuppressive agent.

95. The method of claim 94, wherein the immunosuppressive agent is cyclosporine, FK506, rapamycin, methotrexate, cyclophosphamide, or prednisone.

96. A kit comprising in a first container, an anti-CD20 antibody, and in a second  
10 container, a cytotoxic agent, wherein the cytotoxic agent has an  $IC_{50}$  of between 40-fold and 4,000-fold less than the  $IC_{50}$  of doxorubicin, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, and wherein the  $IC_{50}$  of each of the cytotoxic agent and doxorubicin is measured by a method comprising:

- 15 (a) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the cytotoxic agent for a 72- to 96-hour period;
- (b) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of doxorubicin for a 72- to 96-hour period; and
- 20 (c) identifying a concentration of the cytotoxic agent and doxorubicin, respectively, at which 50% fewer cells in the CD20-expressing cell populations, respectively, are viable at the end of the period relative to a CD20-expressing cell population cultured in the absence of the cytotoxic agent and doxorubicin,

25 wherein the CD20-expressing cell populations of steps (a), (b) and (c) are of the same cell type and are cultured under the same conditions,

and wherein the concentration of the cytotoxic agent and doxorubicin identified in step (c) is the  $IC_{50}$  of the cytotoxic agent and doxorubicin, respectively.

97. The kit of claim 96, wherein the kit further comprises, in a third container, a  
30 linker for conjugating the anti-CD20 antibody to the cytotoxic agent.

98. A kit comprising in a first container, an anti-CD20 antibody, and in a second container, a cytotoxic agent, wherein upon conjugation of the anti-CD20 antibody and the drug, the resulting conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an unconjugated  
35 form of the anti-CD20 antibody in the CD20-expressing cell, with the proviso that the



cytotoxic agent is not a radioisotope or a toxin, and wherein the rates of accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

- 5 (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the unconjugated antibody, wherein the populations of steps (a) and (b) are cultured under the same conditions; and
- 10 (c) measuring the amount of the conjugate and unconjugated antibody accumulated in the populations of steps (a) and (b), respectively.

99. A kit comprising in a first container, an anti-CD20 antibody, and in a second container, a cytotoxic agent, wherein upon conjugation of the anti-CD20 antibody and the drug, the resulting conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an anti-CD20 antibody-doxorubicin conjugate in a CD20-expressing cell of the same cell type, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, and wherein the rates of accumulation of the anti-CD20 antibody-cytotoxic agent conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-cytotoxic agent conjugate;
- (b) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate, wherein the populations of steps (a) and (b) are cultured under the same conditions; and
- 25 (c) measuring the amount of the anti-CD20 antibody-cytotoxic agent conjugate and anti-CD20 antibody-doxorubicin conjugate accumulated in the populations of steps (a) and (b), respectively.

100. A kit comprising in a first container, an anti-CD20 antibody, and in a second container, a cytotoxic agent, wherein upon conjugation of the anti-CD20 antibody and the drug, the resulting conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the accumulation of

the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the conjugate;
- 5 (b) culturing a population of the CD20-expressing cell with the unconjugated form of the anti-CD20 antibody; and
- (c) detecting by confocal fluorescence microscopy localization of the conjugate and the unconjugated form of the anti-CD20 antibody in the populations of steps (a) and (b), respectively,

10 wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the CD20-expressing cell if:

- 15 (i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the unconjugated form of the antibody in a non-peripheral region; or
- 20 (ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the majority of CD20-expressing cells of the population of step (b).

101. A kit comprising in a first container, an anti-CD20 antibody, and in a second  
25 container, a cytotoxic agent, wherein upon conjugation of the anti-CD20 antibody and the drug, the resulting conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an anti-CD20 antibody-doxorubicin conjugate, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate  
30 and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, in the CD20-expressing cell, wherein the accumulation of the conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the conjugate;

- (b) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate; and
- (c) detecting by confocal fluorescence microscopy localization of the conjugate and the anti-CD20 antibody-doxorubicin conjugate in the populations of steps (a) and (b), respectively,

wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the CD20-expressing cell if:

- (i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the anti-CD20 antibody-doxorubicin conjugate in a non-peripheral region; or
- (ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the majority of CD20-expressing cells of the population of step (b).

102. A kit comprising in a first container, an anti-CD20 antibody, in a second container, a cytotoxic agent, and in a third container, a linker for conjugating the anti-CD20 antibody to the cytotoxic agent, wherein upon conjugation of the anti-CD20 antibody and the drug via the linker, the resulting conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, and wherein the rates of accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the unconjugated antibody, wherein the populations of steps (a) and (b) are cultured under the same conditions; and
- (c) measuring the amount of the conjugate and unconjugated antibody accumulated in the populations of steps (a) and (b), respectively.

103. A kit comprising in a first container, an anti-CD20 antibody, in a second container, a cytotoxic agent, and in a third container, a linker for conjugating the anti-CD20 antibody to the cytotoxic agent, wherein upon conjugation of the anti-CD20 antibody and the drug via the linker, the resulting conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

- 10 (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the unconjugated form of the anti-CD20 antibody; and
- 15 (c) detecting by confocal fluorescence microscopy localization of the conjugate and the unconjugated form of the anti-CD20 antibody in the populations of steps (a) and (b), respectively,

wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the CD20-expressing cell if:

- (i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the unconjugated form of the antibody in a non-peripheral region; or
- 25 (ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the majority of CD20-expressing cells of the population of step (b).

104. A kit comprising in a first container, an anti-CD20 antibody, in a second container, a cytotoxic agent, and in a third container, a linker for conjugating the anti-CD20 antibody to the cytotoxic agent, wherein upon conjugation of the anti-CD20 antibody and the drug via the linker, the resulting conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than

the accumulation of an anti-CD20 antibody-doxorubicin conjugate, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, in the CD20-expressing cell, wherein the accumulation of the conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the conjugate;
- (b) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate; and
- (c) detecting by confocal fluorescence microscopy localization of the conjugate and the anti-CD20 antibody-doxorubicin conjugate in the populations of steps (a) and (b), respectively,

wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the CD20-expressing cell if:

- (i) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of the population of step (b) contain the anti-CD20 antibody-doxorubicin conjugate in a non-peripheral region; or
- (ii) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the majority of CD20-expressing cells of the population of step (b).

105. A kit comprising in a first container, an anti-CD20 antibody, in a second container, a cytotoxic agent, and in a third container, a linker for conjugating the anti-CD20 antibody to the cytotoxic agent, wherein upon conjugation of the anti-CD20 antibody and the drug via the linker, the resulting conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an anti-CD20 antibody-doxorubicin conjugate in a CD20-expressing cell of the same cell type, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin

conjugate comprise the same anti-CD20 antibody, and wherein the rates of accumulation of the anti-CD20 antibody-cytotoxic agent conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- (a) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-cytotoxic agent conjugate;
- (b) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate, wherein the populations of steps (a) and (b) are cultured under the same conditions; and
- (c) measuring the amount of the anti-CD20 antibody-cytotoxic agent conjugate and anti-CD20 antibody-doxorubicin conjugate accumulated in the populations of steps (a) and (b), respectively.

106. A kit comprising:

- (a) an anti-CD20 antibody-cytotoxic agent conjugate, wherein the cytotoxic agent of the anti-CD20 antibody-cytotoxic agent conjugate has an  $IC_{50}$  of between 40-fold and 4,000-fold less than the  $IC_{50}$  of doxorubicin, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, and wherein the  $IC_{50}$  of each of the cytotoxic agent and doxorubicin is measured by a method comprising:
  - (i) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the cytotoxic agent for a 72- to 96-hour period;
  - (ii) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of doxorubicin for a 72- to 96-hour period; and
  - (iii) identifying a concentration of the cytotoxic agent and doxorubicin, respectively, at which 50% fewer cells in the CD20-expressing cell populations of steps (i) and (ii), respectively, are viable at the end of the period relative to a CD20-expressing cell population cultured in the absence of the cytotoxic agent and doxorubicin,

wherein the CD20-expressing cell populations of steps (i), (ii) and (iii) are of the same cell type and are cultured under the same conditions,

and wherein the concentration of the cytotoxic agent and doxorubicin identified in step (iii) is the  $IC_{50}$  of the cytotoxic agent and doxorubicin, respectively, and

- (b) a notice by a regulatory agency indicating approval for manufacture, use or sale of the conjugate for human administration.

107. A kit comprising:

- (a) an anti-CD20 antibody-cytotoxic agent conjugate, wherein the anti-CD20 antibody-cytotoxic agent conjugate has an  $IC_{50}$  of between 40-fold and 4,000-fold less than the  $IC_{50}$  of an anti-CD20 antibody-doxorubicin conjugate, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, and wherein the  $IC_{50}$  of each of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate is measured by a method comprising:

- (i) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the anti-CD20 antibody-cytotoxic agent conjugate for a 72- to 96-hour period;
- (ii) culturing one or more CD20-expressing cell populations in the presence of one or more concentrations of the anti-CD20 antibody-doxorubicin conjugate for a 72- to 96-hour period; and
- (iii) identifying a concentration of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate, respectively, at which 50% fewer cells in the CD20-expressing cell populations of steps (i) and (ii), respectively, are viable at the end of the period relative to a CD20-expressing cell population cultured in the absence of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate,

wherein the CD20-expressing cell populations of steps (i), (ii) and (iii) are of the same cell type and are cultured under the same conditions,

and wherein the concentration of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate identified in step (iii) is the  $IC_{50}$  of the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate, respectively; and

- (b) a notice by a regulatory agency indicating approval for manufacture, use or sale of the conjugate for human administration.

108. A kit comprising:

- (a) an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, and wherein the rates of accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:

- (i) culturing a population of the CD20-expressing cell with the conjugate;
- (ii) culturing a population of the CD20-expressing cell with the unconjugated antibody, wherein the populations of steps (i) and (ii) are cultured under the same conditions; and
- (iii) measuring the amount of the conjugate and unconjugated antibody accumulated in the populations of steps (i) and (ii), respectively; and

- (b) a notice by a regulatory agency indicating approval for manufacture, use or sale of the conjugate for human administration.

109. A kit comprising:

- (a) an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate has a rate of accumulation in a CD20-expressing cell that is between 20-fold and 5,000-fold greater than the rate of accumulation of an anti-CD20 antibody-doxorubicin conjugate in a CD20-expressing cell of the same cell type, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, and wherein the rates of accumulation of the anti-CD20 antibody-cytotoxic agent conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- (i) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-cytotoxic agent conjugate;



- (ii) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate, wherein the populations of steps (i) and (ii) are cultured under the same conditions; and
- 5 (iii) measuring the amount of the anti-CD20 antibody-cytotoxic agent conjugate and anti-CD20 antibody-doxorubicin conjugate accumulated in the populations of steps (i) and (ii), respectively; and
- 10 (b) a notice by a regulatory agency indicating approval for manufacture, use or sale of the conjugate for human administration.
110. A kit comprising:
- (a) an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold
- 15 greater than the accumulation of an unconjugated form of the anti-CD20 antibody in the CD20-expressing cell, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the accumulation of the conjugate and of the unconjugated form of the antibody are measured by a method comprising:
- 20 (i) culturing a population of the CD20-expressing cell with the conjugate;
- (ii) culturing a population of the CD20-expressing cell with the unconjugated form of the anti-CD20 antibody; and
- 25 (iii) detecting by confocal fluorescence microscopy localization of the conjugate and the unconjugated form of the anti-CD20 antibody in the populations of steps (a) and (b), respectively,
- wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater
- 30 than the accumulation of the unconjugated form of the anti-CD20 antibody in the CD20-expressing cell if:
- (A) between 1.5-fold and 5,000-fold as many cells of the population of step (a) contain a detectable amount of the conjugate in a non-peripheral region as the number of cells of

the population of step (b) contain the unconjugated form of the antibody in a non-peripheral region; or

(B) the accumulation of the conjugate in a non-peripheral region of the majority of CD20-expressing cells of the population of step (a) is between 1.5-fold and 5,000-fold greater than the accumulation of the unconjugated form of the anti-CD20 antibody in the majority of CD20-expressing cells of the population of step (ii); and

(b) a notice by a regulatory agency indicating approval for manufacture, use or sale of the conjugate for human administration.

111. A kit comprising:

(a) an anti-CD20 antibody-cytotoxic agent conjugate, wherein the conjugate exhibits an accumulation in a non-peripheral region inside a CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of an anti-CD20 antibody-doxorubicin conjugate, with the proviso that the cytotoxic agent is not a radioisotope or a toxin, wherein the anti-CD20 antibody-cytotoxic agent conjugate and the anti-CD20 antibody-doxorubicin conjugate comprise the same anti-CD20 antibody, in the CD20-expressing cell, wherein the accumulation of the conjugate and of the anti-CD20 antibody-doxorubicin conjugate are measured by a method comprising:

- (i) culturing a population of the CD20-expressing cell with the conjugate;
- (ii) culturing a population of the CD20-expressing cell with the anti-CD20 antibody-doxorubicin conjugate; and
- (iii) detecting by confocal fluorescence microscopy localization of the conjugate and the anti-CD20 antibody-doxorubicin conjugate in the populations of steps (a) and (b), respectively,

wherein the populations of steps (a) and (b) are cultured under the same conditions and for the same period of time, and wherein the conjugate exhibits an accumulation in the CD20-expressing cell that is between 1.5-fold and 5,000-fold greater than the accumulation of the anti-CD20 antibody-doxorubicin conjugate in the CD20-expressing cell if:

5 (A) between 1.5-fold and 5,000-fold as many cells of the  
population of step (a) contain a detectable amount of the  
conjugate in a non-peripheral region as the number of cells of  
the population of step (b) contain the anti-CD20 antibody-  
doxorubicin conjugate in a non-peripheral region; or  
(B) the accumulation of the conjugate in a non-peripheral  
region of the majority of CD20-expressing cells of the  
population of step (a) is between 1.5-fold and 5,000-fold  
greater than the accumulation of the anti-CD20 antibody-  
doxorubicin conjugate in the majority of CD20-expressing  
10 cells of the population of step (ii); and

(b) a notice by a regulatory agency indicating approval for manufacture,  
use or sale of the conjugate for human administration.

112. The anti-CD20 antibody-cytotoxic agent conjugate of claim 1, 6, 12, 18, 22,  
15 or 26, wherein the conjugate is purified.

113. The pharmaceutical composition of claim 65, 66, 67, 68, 69, or 70, wherein  
the anti-CD20 antibody-cytotoxic agent conjugate is purified.

114. The method of claim 71, 72, 73, 74, 75, 76, 78, 79, 80, 81, 82, or 83, wherein  
the anti-CD20 antibody-cytotoxic agent conjugate is purified.

20 115. The kit as in any one of claims 96-111, wherein the anti-CD20 antibody-  
cytotoxic agent conjugate is purified.

116. The kit as in any one of claims 96-111, wherein the kit further comprises a  
second cytotoxic or a cytostatic agent.

25 117. The kit of claim 116, wherein the second cytotoxic or cytostatic agent is  
selected from the group consisting of an alkylating agent, an anthracycline, an antibiotic, an  
antifolate, an antimetabolite, an antitubulin agent, an auristatin, a chemotherapy sensitizer, a  
DNA minor groove binder, a DNA replication inhibitor, a duocarmycin, an etoposide, a  
fluorinated pyrimidine, a lexitropsin, a nitrosourea, a platinol, a purine antimetabolite, a  
puromycin, a radiation sensitizer, a steroid, a taxane, a topoisomerase inhibitor, a vinca  
alkaloid, a purine antagonist, and a dihydrofolate reductase inhibitor.  
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118. The kit of claim 116, wherein the second cytotoxic or cytostatic agent is  
androgen, anthramycin (AMC), asparaginase, 5-azacytidine, azathioprine, bleomycin,  
busulfan, buthionine sulfoximine, camptothecin, carboplatin, carmustine (BSNU), CC-  
1065, chlorambucil, cisplatin, colchicine, cyclophosphamide, cytarabine, cytidine  
35 arabinoside, cytochalasin B, dacarbazine, dactinomycin (formerly actinomycin),

daunorubicin, decarbazine, docetaxel, doxorubicin, an estrogen, 5-fluorodeoxyuridine, 5-fluorouracil, gramicidin D, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine (CCNU), mechlorethamine, melphalan, 6-mercaptopurine, methotrexate, mithramycin, mitomycin C, mitoxantrone, nitroimidazole, paclitaxel, plicamycin, procarbazine, streptozotocin, tenoposide, 6-thioguanine, thioTEPA, topotecan, vinblastine, vincristine, vinorelbine, VP-16, VM-26, azothioprine, mycophenolate mofetil, methotrexate, acyclovir, gangcyclovir, zidovudine, vidarabine, ribavarin, azidothymidine, cytidine arabinoside, amantadine, dideoxyuridine, iododeoxyuridine, poscarnet, or trifluridine.

119. The kit as in claim 96-111, wherein the kit further comprises a second antibody other than an anti-CD20 antibody.

120. The kit as in claim 119, wherein the second antibody is an anti-CD19 antibody, an anti-CD22 antibody, an anti-CD30 antibody, and an anti-CD40 antibody.

121. The kit as in claim 119, wherein the second antibody is conjugated to a second cytotoxic or cytostatic agent.